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The Interaction of Theophylline with Benzylamine in Aqueous Solution

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The interaction of theophylline with benzylamine in aqueous solution was studied by the solubility method and by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The results show that two types of intearctions, ion pair and stacking, occur between theophylline and benzylamine. Apparent stability constants for the formation of complexes of the ion pair type and stacking type were estimated approximately at 25°C.

Keywords——theophylline; benzylamine; complex formation; ^{1}H-NMR; solubility method; ion pair interaction; stacking interaction

In the previous paper,[1] we reported that theophylline forms a complex with benzylamine in the solid state and suggested that the major driving force is hydrogen bonding between the >NH group and two >C=O groups of theophylline and the $-NH_2$ group of benzylamine. In addition, the interaction between the α,β-unsaturated ketone moiety of theophylline and π electrons of the benzene ring of benzylamine, *i.e.*, stacking effect, was assumed to assist solid complex formation. In this paper we report the results of investigations on the interaction between benzylamine, which has an aliphatic amino group and an aromatic nucleus, and theophylline in aqueous solution. Such a study should clarify what structure of amine is suitable for solubilizing theophylline and should provide fundamental data relevant to the interaction of theophylline with drug molecules.

Experimental

Materials——Theophylline and benzylamine used were those reported previously.[1] Water used was obtained by distilling water purified with ion-exchange resin.

Solubility Method——A 20 ml aliquot of benzylamine solution of appropriate concentration and excess theophylline were taken in a 25 ml test tube. Nitrogen gas was blown through the solution then the test tube was sealed. Test tubes were mounted in water bath thermostated at 25 ± 0.05°C under shaking for 35 h. The subsequent experimental method was the same as that reported previously.[2]

Proton Nuclear Magnetic Resonance (^{1}H-NMR) Spectra——^{1}H-NMR spectra were recorded in deuterium oxide (D_2O) on a Varian XL-200 (200 MHz) spectrometer with tetramethylsilane as an external reference at 25 ± 0.5°C. Bulk susceptibility corrections were tried, but were found to be unnecessary, since the volume magnetic susceptibilities of the samples are equal to that of deuterium oxide for low concentrations of the samples. The chemical shifts were reproducible to better than 0.001 ppm.

The conditions for FT NMR measurements were: spectral width, 2400 Hz; pulse width, 3.0 s (flip angle, about 40°); acquisition time, 3 s; number of data points, 14398; number of transients, 0.2—1 K.

Results and Discussion

Solubility Method

Fig. 1 shows changes in the solubility of theophylline in aqueous solutions containing various concentrations of benzylamine. An increment of solubility S_t shown by equation (1) due to an increase in pH resulting from the addition of benzylamine is considered to be involved in the solubility of theophylline.

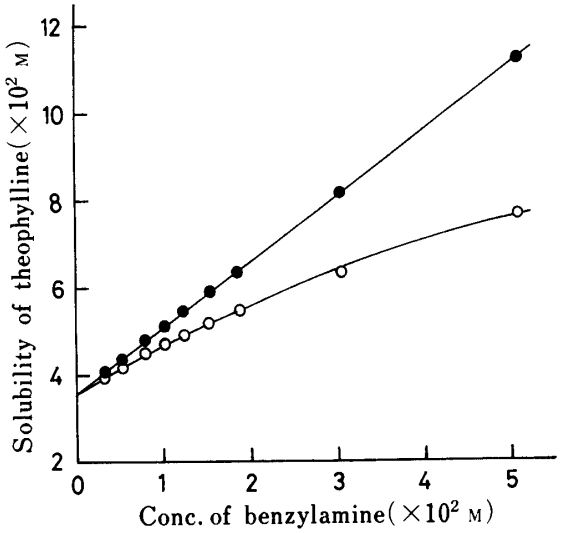

Fig. 1. Solubilization of Theophyline by Benzylamine in Aqueous Solution

—●—: solubility curve of theophylline in the presence of benzylamine.
—○—: calculated solubility curve of theophylline at found pH.

$$S_t = S_0\left\{1 + \frac{K_a}{[H^+]}\right\} \quad (1)$$

where S_0 is the solubility of the unionized species of theophylline, and K_a and $[H^+]$ represent the dissociation constant of theophylline and hydrogen ion concentration, respectively. S_0 and K_a are 3.58×10^{-2} M and 2.29×10^{-9} M at 25°C, respectively.[2] S_t is shown by open circles in Fig. 1. On the other hand, it has been reported that caffeine, having no >NH group at the 7 position does not interact with ethylenediamine[3] and theophylline does not interact with aliphatic monoamines in aqueous solution of pH 5.0,[4] in which theophylline exists as the unionized species. Therefore, the interaction of theophylline with benzylamine does not involve hydrogen bondings between >C=O, >NH and $\overset{+}{>}$N groups of theophylline and $-NH_2$ and $-\overset{+}{N}H_3$ groups of benzylamine. From these results, it is considered that the solubility is increased by the formation of a soluble complex of ion pair type, in addition to an increase in solubilty due to dissociation of a proton from the >NH group of theophylline as reported previously.[2,3] Therefore, we have equation (2).

$$\text{Solubility} = S_t + [\text{complex}] \quad (2)$$

where [complex] represents the concentration of the soluble complex of ion pair type. Assuming that the complex is formed in a molar ratio of 1:1, we determined the apparent stability constant as defined by equation (3) according to the method reported previously.[2]

$$K_{C_{1:1}} = \frac{[T^-BA^+]}{[T^-]\,[BA^+]} \quad (3)$$

where $[T^-BA^+]$, $[T^-]$, and $[BA^+]$ denote the concentrations of the complex of ion pair type, the ionized species of theophylline, and the ionized species of benzylamine, respectively. The results are shown in Table I. In the previous paper[2] a relationship between $K_{C_{1:1}}$ of theophyl-

Table I. Calculated Value of Apparent Stability Constant of Theophyline–Benzylamine Complex in Aqueous Solution at 25°C

Conc. of benzylamine ($\times 10^2$ M)	Solubility of theophylline ($\times 10^2$ M)	pH found	Conc. of ionized theophylline ($\times 10^2$ M)	Conc. of complex ($\times 10^2$ M)	Conc. of ionized benzylamine ($\times 10^2$ M)	$K_{C_{1:1}}$ (M^{-1})
0.308	4.05	7.70	0.411	0.0586	0.244	58.4
0.514	4.36	7.89	0.637	0.138	0.363	59.6
0.822	4.81	8.07	0.965	0.268	0.526	52.7
1.03	5.72	8.15	1.16	0.378	0.611	53.3
1.23	5.42	8.21	1.33	0.511	0.672	57.0
1.54	5.88	8.29	1.60	0.698	0.774	56.4
1.85	6.34	8.36	1.88	0.875	0.882	52.8
3.08	8.17	8.52	2.72	1.87	1.06	65.0
5.14	11.2	8.70	4.11	3.52	1.32	64.9
						(57.8±4.5)[a]

a) The mean standard deviation.

line–monoamine complex and the pK_a of the monoamine was reported. On this basis, $K_{C_{1:1}}$ of benzylamine (pK_a 9.34 at 25°C) was estimated to be about 45 (M^{-1}). The value of $K_{C_{1:1}}$ obtained by the solubility method (Table I) was larger than that estimated from the above relationship. Therefore, the difference (solubility $-S_t$) is considered to reflect the formation of a complex as a result of interaction between benzylamine and theophylline through so-called vertical stacking or plane-to-plane stacking, as seen between theophylline and sodium benzoate,[6] besides the ion pair type complex. To confirm this, the interaction between theophylline and benzylamine was examined in D_2O by ^{1}H-NMR spectroscopy.

^{1}H-NMR Spectra

To reduce the effect of self-association of theophylline,[7] a low concentration of theophylline was used. The ^{1}H-NMR spectrum of 1.00×10^{-3} M theophylline shows signals due to 8-H, 3-Me, and 1-Me at 7.308, 2.856 and 2.653 ppm (from external tetramethylsilane) with a relative intensity of 1 : 3 : 3. Then, various concentrations of 2-methoxyethylamine (2-MEA) from 1.5×10^{-2} to 5.5×10^{-2} M were added to theophylline solution at a constant concentration of 1.00×10^{-3} M and the ^{1}H-NMR signals of theophylline were examined. With an initial increase in concentration of 2-MEA, the signals of 8-H, 3-Me, and 1-Me of theophylline shifted upfield. However, above 2.5×10^{-2} M 2-MEA, the signals hardly shifted further. The order of magnitudes of upfield shifts was 8-H>3-Me>1-Me (Fig. 2). These experimental results can be interpreted as follows; with increase in the concentration of 2-MEA, the pH value of the solution increased, the dissociation of the 7-H proton (>NH group) of theophylline was accelerated to produce $>N^-$ in greater amount and an ion pair type complex involving interaction between the resulting $>N^-$ group and the $-NH_3^+$ group of 2-MEA was produced in greater amount. As a result, the concentration of theophylline containing the $>N^-$ group in the solution (*i.e.*, theophylline having the free $>N^-$ group plus theophylline forming the ion pair type complex) increased and the signal of the adjacent 8-H shifted markedly upfield under the influence of the negative charge of the $>N^-$ group. Further, the signal of 3-Me, to which the electron of $>N^-$ group can transfer more readily through the double bond, shifted more upfield than the signal of 1-Me, to which the electron of the $>N^-$ group cannot readily transfer. Next, benzylamine at the same concentrations of 1.5×10^{-2}—5.5×10^{-2} M as in the case of 2-MEA was added to the solution of theophylline at a constant concentration of 1.00×10^{-3} M, and the ^{1}H-NMR

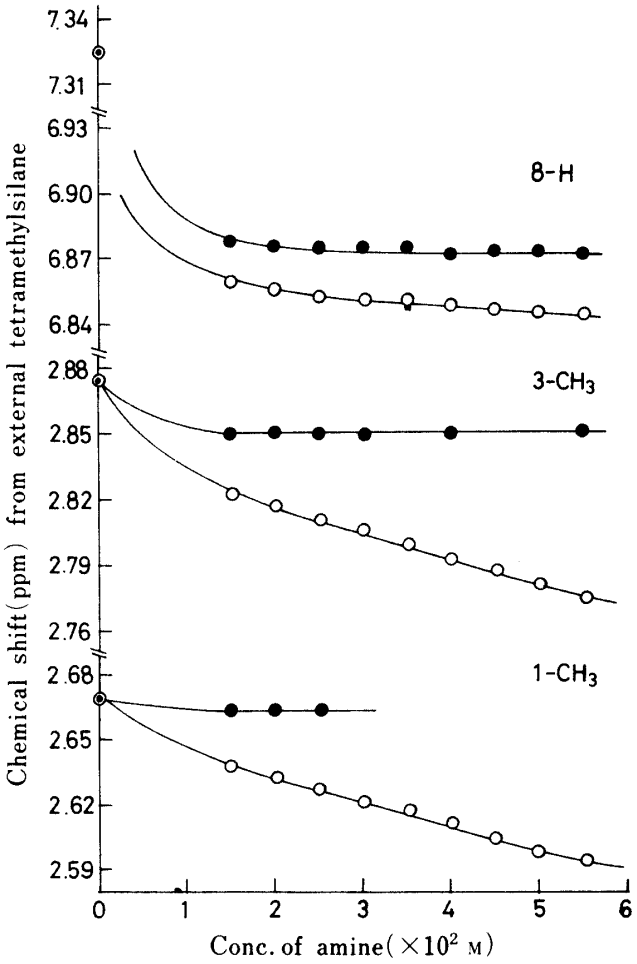

Fig. 2. Chemical Shift Changes for the 8-H, 3-CH_3, and 1-CH_3 Protons of Theophylline as a Function of Amine Concentration

—●— : 2-methoxyethylamine, —○— : benzylamine. Because of overlapping of the signals of 3-CH_3 and 1-CH_3 of theophylline with signals of 2-methoxyethylamine, data could not be obtained in the theophylline-2-methoxyethylamine system.

spectra were examined. The results are shown in Fig. 2. The pK_a values of benzylamine and 2-MEA were similar, being 9.34 and 9.45, respectively at 25°C and the differences in pH values of the solutions containing a constant concentration of theophylline and the same concentrations of these amines were within 0.02 in the concentration ranges used in the present experiment. Therefore, if the concentrations of these two amines are identical, there should be virtually no differences in concentrations of free ionized theophylline (theophylline having the free $>N^-$ group), free unionized theophylline, and the ion pair type complex. Thus, the values obtained by subtracting the upfield shifts of the signals of 8-H, 3-Me, and 1-Me of theophylline in the theophylline-2-MEA system from those in the theophylline–benzylamine system at the same concentration, respectively, may well represent upfield shifts, Δ_{ppm}(Stac), due to a stacking interaction of theophylline and benzylamine. The Δ_{ppm}(Stac) values of 8-H, 3-Me, and 1-Me are plotted against the concentration of benzylamine in Fig. 3. The Δ_{ppm}(Stac) values for 8-H, 3-Me, and 1-Me increased with increase in the concentration of benzylamine. Namely, it was found that a stacking interaction occurred between theophylline and benzylamine in aqueous solution in addition to the ion pair type interaction and the stacking interaction increased with increase in the concentration of benzylamine. The value of Δ_{ppm}(Stac) of 3-Me was slightly larger than that of 1-Me, and that of 1-Me was larger than that of 8-H. The 3-Me group is nearer to the center of the benzene ring of benzylamine than 1-Me and the latter is nearer than 8-H. The apparent stability constant $K_{C_{1:1}}$(Stac) of the complex due to stacking was calculated using equation (4) according to the procedure of Hanna and Ashbaug,[8] assuming a 1:1 complex.

$$\frac{1}{\Delta_{obsd}} = \frac{1}{K(\delta_c - \delta_a)C_b} + \frac{1}{\delta_c - \delta_a} \quad (4)$$

K: $K_{C_{1:1}}$(Stac).
C_b: concentration of benzylamine.
δ_c: chemical shift of theophlline proton in the stacking complex.
δ_a: chemical shift of theophylline in nncomplexed form.
Δ_{obsd}: the difference between observed chemical shift(Stac) and δ_a, Δ_{ppm}(Stac).

The apparent stability constants $K_{C_{1:1}}$ (Stac) determined by using the date for 1-Me and 3-Me were 7.1 and 7.4 M^{-1}, respectively. The Δ_{obsd}, namely, Δ_{ppm}(Stac) of 8-H was too small to

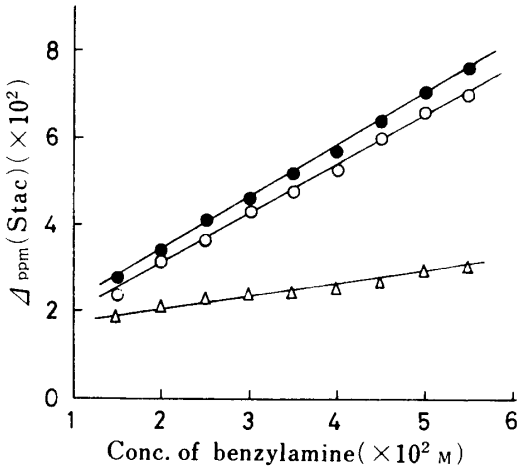

Fig. 3. Chemical Shift Changes for the 8-H, 3-CH_3 and 1-CH_3 Protons of Theophylline induced by Stacking Interraction as a Function of Benzylamine Concentration

—●— : 3-CH_3, —○— : 1-CH_3, —△— : 8-H.

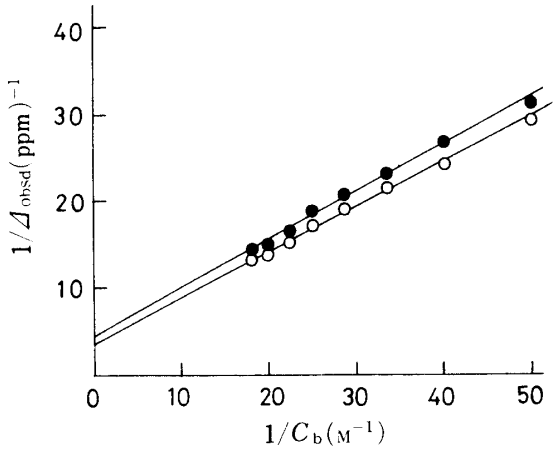

Fig. 4. Plot of $\Delta_{obsd}^{-1}\{=\Delta_{ppm}(Stac)^{-1}\}$ Values of Theophylline Methyl Groups *vs.* Reciprocal of Benzylamine Concentration

—●— : 3-CH_3, —○— : 1-CH_3.

allow the determination of $K_{C_{1:1}}$ (Stac). Average $K_{C_{1:1}}$ (Stac) was 7.2 M^{-1} (Fig. 4). Since $K_{C1:1}$ (Stac) was larger than the values[6,9] reported for the stacking interaction of xanthine with sodium benzoate in aqueous solution, it is presumed that the stacking interaction is enhanced by ion pair type interaction. The stacking interaction is considered to involve unionized theophylline–unionized benzylamine, unionized theophylline–ionized benzylamine, ionized theophylline–unionized benzylamine, and also ionized theophylline–ionized benzylamine.

From the results described above, it is considered that theophylline and benzylamine undergo both ion pair and stacking interaction. It is probably because the solubility of theophylline increases as a result of the formation of a soluble complex by stacking that apparent stability constant $K_{C_{1:1}}$ obtained by the solubility method is larger than the value expected from the pK_a of benzylamine.

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